REMARKS

Claims 1, 3, 4, 7-13 and 16-20 are presently. In the instant Amendment, Claim 11 has been canceled, without prejudice, and Claims 1 and 18 have been amended. Support for amended Claims 1 and 18 can be found generally throughout the instant Application, and in particular on pages 10-11 and Claims 1-18 as originally filed.

The Invention is Nonobvious

Claims 1, 3, 4, 7-13, 16-17, and 19-20 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Keating *et al.* in view of Brown *et al.* and in view of US published application 20050118690 (the '690 application) and in further view of Yang *et al.*. The Examiner has asserted Applicant claims a method of identifying an agent that modulates the activity of a target molecule wherein the agent contacts a cell and modulates the target molecule, and wherein the cell also comprises two reporter genes. The Examiner believes that after contact by the agent, cell propagation and reporter activity are measured. One of the reporter genes produces an enzyme, and the substrate of the enzyme is added after a delay, specifically at least two cell cycles. The Examiner contends that measuring reporter activity comprises disrupting the cell by permeabilizing the membrane, or destroying the membrane, and that the target molecule affects the reporter gene, and is further limited to a heterologous molecule and can be a nucleic acid or polypeptide. The Examiner also contends the target molecule affects cellular propagation indirectly or through an intermediary molecule, and that the target molecule can also affect the reporter gene and cellular propagation directly. The Examiner also believes the

reporter gene produces an enzyme whose activity is detectable on the basis of conversion of a substrate, and that the cell is a yeast cell, specifically *S. cerevisiae*.

The Examiner has also asserted that Keating *et al.*, specifically in the introduction and the Materials and Methods, teach a method of identifying an agent that modulates the activity of a target molecule by contacting a cell and modulating a target molecule, wherein an agent, EGF, modulates a target molecule, ATM (a heterologous kinase), which induces a luciferase reporter. The Examiner believes ATM is known to be involved in cell cycle control. It is also the Examiner's position that Keating *et al.* teach that cells were incubated with EGF for 16 hours before cells extracts were prepared, and that EGF was added during the log phase, therefore at least one or two cell cycles have occurred. The Examiner further contends Keating *et al.* teach that firefly luciferase substrate (LARII) was added and reporter activity was measured using a Dual Luciferase Assay. The Examiner has admitted though that Keating *et al.* do not teach the use of two different reporters or improving signal-to-background ratio.

The Examiner further believes that Brown *et al.* teach a dual reporter assay for evaluating chimeric yeast/mammalian Gα proteins in *S. cerevisiae*. The Examiner has asserted that Gα proteins can modulate effectors to cause signal propagation, and that GPCRs can also directly affect propagation. The Examiner believes Table 3 of Brown *et al.* lists the different concentrations of agonists used to determine the effect on the pherómone response pathway, and that the two reporter constructs used were FUS1-HIS3 and FUS-LacZ. In the Examiner's opinion, page 10 of the instant Specification states that when these two constructs are combined, the improved signal-to-background ratio is 100-150:1, a beta-galactosidase assay is performed

with CPRG as the substance to measure activity, that CPRG is converted to chlorophenol red after 24 hours of incubation, cell growth is determined, and that cells were also disrupted to perform a Western blot, and Glass beads are used to disrupt the membrane.

The Examiner has also asserted that Yang *et al.* teach a dual fluorescent assay for improving signal-to-background ratio, specifically at pages 8212, 8214, and 8216. In particular, the Examiner believes Yang *et al.* teach optimizing GFP and BFP to enhance expression levels.

In light of the Examiner's interpretations of the references discussed above, and the instant Specification, it is the position of the Examiner that the ordinarily skilled artisan, desiring to use a dual reporter system to improve signal-to-background ratio, would have been motivated to combine the teachings of Keating *et al.*, who the Examiner believes teach a method of identifying an agent that modulates the activity of a target molecule, which induces a reporter and delays adding the substrate of the reporter, with the teachings of Brown *et al.*, who the Examiner believes teach a dual reporter system with HIS3 as the first reporter and lacZ as the second reporter, and with the teachings of Yang *et al.*, who the Examiner believes teach a dual reporter system for improving signal-to-noise ratio because, in the Examiner's opinion, Yang *et al.* teach that enhancing optimization of two reporters allows for maximum signal intensity.

Furthermore, the Examiner believes it would have been obvious to one of ordinary skill in the art to improve signal-to-background ratio because, in the Examiner's opinion, higher expression yields provide greater sensitivity. The Examiner further contends that, given the teachings of the prior art and the level of the ordinary skilled artisan at the time of Applicant's Invention, it must be considered, absent evidence to the contrary, that the skilled artisan would

have had a reasonable expectation of success in practicing the claimed Invention.

This rejection is respectfully traversed. In the instant Amendment, Claim 1 has been amended to be directed towards, *inter alia*, a method utilizing a double reporter assay for improving signal-to-background ratio to identify an agent which modulates activity of a target molecule, wherein said target molecule affects cellular propagation, said method comprising the steps of:

a] contacting a cell with a candidate compound, wherein said cell comprises said target molecule, and wherein said cell further comprises a growth marker reporter gene and a gene coding for enzyme whose activity is detectable on the basis of conversion of a substrate;

b] adding said substrate and a substance capable of permeabilizing the membrane of said cell with a delay after said contacting step a].

In addition, Claim 18 has been amended to be directed towards, *inter alia*, a method of identifying an agent which modulates the activity of at least one target molecule, wherein said at least one target molecule affects cellular propagation, said method comprising the steps of:

(a) contacting a first cell with a candidate compound, wherein said first cell comprises a first target molecule, and wherein said cell further comprises a growth marker reporter gene and a gene coding for enzyme whose activity is detectable on the basis of conversion of a substrate;

(b) contacting a second cell with a candidate compound, wherein said second cell comprises a second target molecule, and wherein said cell further comprises a growth

marker reporter gene and a gene coding for enzyme whose activity is detectable on the basis of conversion of a substrate;

(c) adding said substrate and a substance capable of permeabilizing the membrane of said cell with a delay after said contacting steps (a) and (b).

Support for amended Claim 1 as well as for amended Claim 18 can readily be found on pages 10-11 of the instant Specification.

It is respectfully submitted that amended Claims 1 and 18 are clearly nonobvious in light of the references the Examiner has cited in making this rejection, either alone or in combination. In making this rejection, the Examiner has asserted that Brown *et al.* teach a dual reporter assay for evaluating chimeric yeast/mammalian Gα proteins in *S. cerevisiae*. However, as explained in the instant Specification, there are substantial differences between the instant Invention and the teachings of Brown *et al.*. In particular, on page 10, lines 30-31 and page 11, lines 1-2 of the instant Specification, it is acknowledged that Brown *et al.* utilize a double reporter gene assay and that FUS1-HIS3 and FUS1-lacZ are utilized simultaneously in a β-galactosidase liquid assay using CPRG as substrate. However, in lines 2-10 of page 11, the dramatic differences between the instant Invention and Brown *et al.* are discussed. Indeed, it is specifically explained that:

> CPRG is added here [in Brown *et al.*] during the entire period of stimulation of the receptor with ligand. In contrast, CPRG is added in this method [of the instant Invention] ***together with a detergent*** in buffered solution only after stimulation of the receptor with ligand, resulting in a marked improvement of the β-galactosidase measurement. For if, on the one hand, CPRG is present during ligand-induced growth, the latter is readily inhibited, and, on the other hand, ***CPRG can reach the cell interior through the plasma membrane only with difficulty. Both problems are avoided if CPRG is added <u>together with a detergent capable of disrupting the plasma membrane</u>, only after growth has finished.***

(p. 11, lines 2-10 of the instant Specification (emphasis added)).

None of the references the Examiner has attempted to combine with the teachings of Brown *et al.* teach that a substrate is added concurrently with substance capable of permeabilizing the membrane of said cell with a delay after said contacting step. Indeed, as the Examiner has admitted, Keating *et al.* do not even teach a dual promoter assay, let alone the addition of a permeabilizing substance such as detergent. Moreover, Yang *et al.* teach nothing with respect to a method for identifying an agent which modulates activity of a target molecule, wherein the target molecule affects cellular propagation. Rather, Yang *et al.* teach variants of GFP. Moreover, contrary to the instant Invention, which teaches *inter alia*, adding the substrate and a substance capable of permeabilizing the membrane are added with a delay after contacting the cell with the candidate compound, Yang is unconcerned with rupturing and killing the cell, and indeed, touts that the GFPs have an advantage over other reporter proteins in that they can be used in a ***living*** organism. In particular, on page 8212, Yang *et al.* specifically state:

> The principal advantage of GFP reporter systems is the ability to detect fluorescence in ***living*** specimens with real-time kinetics (emphasis added).

Since Brown *et al.* teach nothing with respect to a method in which a substance capable of permeabilizing a cell's membrane to rupture the cell is added, and Yang *et al.* tout the advantage of their GFP variants is that they have applications in ***living*** organisms, it is respectfully submitted that there is no teaching or suggestion in any of these references that motivate one of ordinary skill in the art to combine their teachings as the Examiner has done in making this rejection. On the contrary, it appears Applicants' disclosure provided such

motivation in making this rejection. However, the Examiner cannot rely on impermissible hindsight to arrive at a determination of obviousness. *In re Fritch*, 23 U.S.P.Q.2d 1780, 1784 (Fed. Cir. 1992). The Court of Appeals for the Federal Circuit has stated that "selective hindsight is no more applicable to the design of experiments than it is to the combination of prior art teachings. There must be a reason or suggestion in the art for selecting the procedure used, other than the knowledge learned from the Applicant's disclosure." *Interconnect Planning Corporation v. Feil.*, 227 U.S.P.Q. 543, 551 (Fed. Cir. 1985)]. *In re Dow Chemical Co.*, 5 U.S.P.Q.2d 1529, 1532 (Fed. Cir. 1988).

Furthermore, Claim 18 has been rejected under 35 U.S.C. § 103(a) as being unpatentable over Crossin *et al.* in view of Keating *et al.* in view of US Patent 6,063,578 and further in view of U.S. Published Application 20050118690. The Examiner has asserted that what is claimed is a method for identifying an agent that modulates the activity of a target molecule, wherein the agent contacts a cell and modulates the target molecule, and wherein the cell also comprises two reporter genes. The Examiner believes that after contact by the agent, cell propagation and reporter activity are measured, and that the reporter genes produce a growth market reporter and a reporter that is an enzyme. The Examiner contends that measuring reporter activity comprises disrupting the cell by permeabilizing the membrane, or destroying the membrane. It is also the Examiner's belief that a second cell is claimed with a target molecule and a reporter gene, and that after contact by the agent, cell propagation and reporter activity are measured, and the substrate of the enzyme is added after a delay.

In the Examiner's opinion, Crossin *et al.* teach a method of identifying an agent that

modulates the activity of a target molecule by contacting a cell and modulating a target molecule, wherein an agonist, N-CAM, modulates a target molecule, GRE, which induces a luciferase reporter. Also, the Examiner has asserted that N-CAM inhibits cell proliferation, and that in measuring luciferase activity, cells were lysed. The Examiner also has asserted that Crossin *et al.* teach a second cell with a second target molecule, CM-V and a second reporter beta-galactosidase. In the Examiner's opinion, N-CAM the agonist. The Examiner has admitted though that Crossin *et al.* do not teach the use of two reporters or adding the substrate after a delay.

Furthermore, the Examiner has asserted Keating *et al.* (specifically, the introduction at p. 4282 and Materials & Methods, 1st and 6th paragraphs) teach a method of identifying an agent that modulates the activity of a target molecule by contacting a cell and modulating a target molecule, wherein an agent, EGF, modulates a target molecule, ATM (a heterologous kinase), which induces a luciferase reporter. The Examiner believes ATM is known to be involved in cell cycle control (see Abstract and Introduction). The Examiner contends that Keating *et al.* teach incubating cells with EGF for 16 hours before cell extracts were prepared, and that EGF was added during log phase and thus, at least one or two cell cycles have occurred. The Examiner has also asserted that firefly luciferase substrate (LARII) was added and reporter activity was measured using a Dual Luciferase Assay. However, the Examiner has admitted that Keating *et al.* do not teach the use of two different reporters or improving signal-to background ratio.

In addition, the Examiner has asserted the '578 patent (specifically columns 8-10) teach a dual reporter assay, and that two different reporters need to be used. The Examiner also contends

that the '578 patent teaches enzymatic and fluorescent proteins, and that the precise reporter genes used are not critical as long as expression can be detected.

The Examiner also believes that the '690 application (specifically paragraphs 92 and 93) teach a dual reporter assay for isolating transformants, and that it is preferable to have two reporter genes within the cell, wherein one reporter gene, when expressed, provides a growth advantage to transformed cells that are expressing the variant regulator protein, like LEU2, HIS3, LYS2, TRP1, URA3 or ADE2, which allows for the isolation of such tranformants through selected pressures. It is also the Examiner's opinion that the other reporter gene provides a colormetric marker, such as the lacZ gene and its encoded protein, beta-galactosidase and that alternatively, the second reporter provides a fluorescent or luminescent marker, such as GFP.

In light of the Examiner's interpretations of the instant Application and the teachings of references discussed above, it is the opinion of the Examiner that an ordinary skilled artisan, desiring to use a dual reporter system, would have been motivated to combine the teachings of Crossin *et al.*, which the Examiner believes teach a method of identifying an agent that modulates the activity of a target molecule, which induces a reporter, with the '690 application teaching a dual reporter system because, in the Examiner's opinion, the '578 patent teaches that the dual reporter system allows for observation of more than one charge induced by a candidate agent. The Examiner asserts that, for example, one reporter can indicate there is a change in transcription. Thus, in the Examiner's opinion, it would have been obvious to one of ordinary skill in the art to use dual reporters because the Examiner believes both processes occur on the same molecule, which the Examiner contends more accurately reflects the natural environment.

Moreover, the Examiner believes that given the Examiner's interpretation of the teachings of the prior art and the level of the ordinarily skilled artisan at the time of the instant Invention, the Examiner has asserted it must be considered, absent evidence to the contrary, that the skilled artisan would have had a reasonable expectation of success in practicing the instant Invention.

This rejection is respectfully traversed. As explained above, Claim 18 is directed towards, *inter alia*, a method of identifying an agent which modulates the activity of at least one target molecule, wherein said at least one target molecule affects cellular propagation, said method comprising the steps of:

(a) contacting a first cell with a candidate compound, wherein said first cell comprises a first target molecule, and wherein said cell further comprises a growth marker reporter gene and a gene coding for enzyme whose activity is detectable on the basis of conversion of a substrate;

(b) contacting a second cell with a candidate compound, wherein said second cell comprises a second target molecule, and wherein said cell further comprises a growth marker reporter gene and a gene coding for enzyme whose activity is detectable on the basis of conversion of a substrate;

(c) adding said substrate and a substance capable of permeabilizing the membrane of said cell with a delay after said contacting steps (a) and (b).

Moreover, as explained above, on page 11 of the instant Specification, it is clearly explained that the substrate CPRG is added ***together with a detergent*** in buffered solution only after stimulation of the receptor with the ligand. Yet, Crossin *et al.* teach that the cell is first lysed ***and then*** the luciferase substrate is added. Indeed, on page 2688, right column, Crossin *et*

al. teach:

> Cells were washed twice with PBS and solubilized with 150 µl of lysis buffer (100 mM Tris-acetate, pH 7.8./10 mM magnesium acetate/1% Triton-X 100/1 mM EDTA/1 mM DTT). Cell lysates were cleared by centrifugation at 15,000 RPM at 4°C for 10 min. Luciferase assays were performed on 20 µl of cell lysate using an LB 96 P MicroLumat fluorometer (EG&G Berthold/Wallace, Gaithersburg, MD). Lysates were mixed with reaction buffer (66 µM D-luciferin potassium salt/2 mM ATP/ 100 mM Tris-acetate, pH 7.8/10 mM magnesium acetate/1 mM EDTA) and the fluorescence intensity was measured. To normalize for transfection efficiency, 20-µl aliquots of cell extract were assayed for β-gal activity using the FluoReporter *lacZ*/Galactosidase kit (Molecular Probes). Levels of activity were quantitated in a cytoFluor 2350 Fluorescence Measurement System (Millipore). The luciferase activity of each sample was normalized to an internal reference standard of β-gal activity)

This passage makes clear that, contrary to amended Claim 18, in which the substrate is added after the cells are contacted with a compound, Crossin *et al.* teach the substrate is never added to the cells. Rather, the cells are lysed, the lysate collected and then the substrate is added. None of the other references the Examiner has cited in this rejection teach to the contrary Hence, amended Claim 18 is clearly nonobvious in light of the cited references, either alone or in combination, and this rejection should be withdrawn.

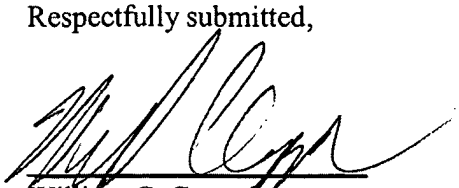
Fees

No fees are believed to be necessitated by the instant response. However, should this be in error, authorization is hereby given to charge Deposit Account no. 18-1982 for any underpayment, or to credit any overpayments.

CONCLUSION

Applicants respectfully request entry of the foregoing amendments and remarks in the file history of the instant Application. The Claims as amended are believed to be in condition for allowance, and reconsideration and withdrawal of all of the outstanding rejections is therefore believed in order. Early and favorable action on the claims is earnestly solicited.

Respectfully submitted,



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